Iowa State University

Digital Repository

Creative Components

Iowa State University Capstones, Theses and Dissertations

Fall 2020

Manganese and glyphosate effects on yield of glyphosate resistant soybean

Adam Shafer

Manganese and Glyphosate Effects on Yield of Glyphosate Resistant Soybean

By

Adam Shafer

A creative component submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Agronomy

Program of Study Committee

Antonio P. Mallarino (Major Professor)

Allen D. Knapp

Iowa State University

Ames, Iowa

2020

TABLE OF CONTENTS

INTRODUCTION

Plants require seventeen different nutrients to survive and thrive, referred to as essential nutrients. To be considered essential, a nutrient must be directly linked to plants nutrition, cannot be replaced or substituted within the plant by other nutrients, and must be necessary for the completion of the plant life cycle. These nutrients are often classified as macronutrients and micronutrients, based on the nutrient amount required by the plant. Of the seventeen essential nutrients, nine are considered macronutrients, and eight are considered micronutrients. There is no widely accepted definition, however, and some macronutrients are referred to as secondary nutrients. Although macronutrients are needed by the plant at much higher quantities, micronutrients are gaining more attention in row crop production, as higher yields are sought after. Essential micronutrients include boron (B), chlorine (Cl), cobalt (Co), copper (Cu), iron (Fe), Manganese (Mn), molybdenum (Mo), and zinc (Zn). nickel (Ni) recently began to be considered essential, mainly for legumes due to its role at metabolizing nitrogen compounds resulting from symbiotic dinitrogen fixation.

The concentration and plant availability of micronutrients in soils are influenced by several factors. A soil organic matter content, texture, and parent material mineralogy can be directly linked to concentration in the soil of micronutrients (Morvedt et al., 1991). The US Geological Survey conducted research to find areas of high and low soil micronutrient concentrations (Shacklette and Boerngen, 1984), but this information was of limited value for crop production because measurements were done for total soil content rather than for plant available amounts. Plant availability of several of these elements is greatly impacted by soil temperature, moisture, and aeration (Mortvedt et al., 1991; Mengel et al., 2001). These environmental factors interacting with activity of soil microorganisms, to a large extent determine the plant available levels of micronutrients in soils by mediating complex chemical and biological processes. In soybean production areas across the North Central region, many of these micronutrients are not commonly found to be deficient, except in uncommon sandy soils. In the region, mainly Indiana and Michigan, Mn deficiencies have been reported in soils with finer texture and more organic matter. Iron deficiency chlorosis (IDC) in soybean is common in high-pH, calcareous soils west of the Mississippi River because calcium carbonates reduce the plant availability of Fe (Mallarino et al., 2017).

Soybean is particularly sensitive to low levels of Mn and Fe, and much more sensitive than corn or small grains (Scott and Aldrich, 1970; Mengel et al., 2001). Plants absorb the ion form of Mn (Mn^{2+}) and other complexed forms. Manganese is involved in many plant metabolic processes, such as the water-splitting reaction in Photosystem II, detoxification of reactive oxygen species, cofactor of many enzymes, and deposition of cuticular waxes in leaves among others (Alejandro et al., 2020). Soybean exhibits Mn deficiency as interveinal chlorosis in the leaves. Manganese deficiencies are commonly found in calcareous or organic soils. As soil pH level increases above neutral (7.0), Mn availability decreases. On the other hand, Mn levels can become toxic at very low pH levels (Mengel et al., 2001).

Research has shown that when the Mn soil supply is deficient, Mn application to the soil or foliage can increase soybean yield. Randall et al. (1975) conducted one of the most complete and cited research on Mn in soybean in the North Central region; the experiments were conducted on two Wisconsin fields with histories of soybean Mn deficiency symptoms. Two trials evaluated several rates of $MnSO_4$ broadcasted before planting soybean, $MnSO_4$ or Mn-

EDTA (ethylenediaminetetraacetic acid) banded with the planter, and either source sprayed to the foliage once or twice from pre-bloom to early bloom stages (no better-defined soybean growth stage was provided). Two other trials evaluated time and frequency of foliar application using one rate of Mn-EDTA sprayed during the aforementioned growth stages. Results of these four trials showed that all Mn sources and methods of application increased soybean yield except for Mn-EDTA applied to the soil, and that banded $MnSO_4$ with the planter was more efficient than broadcast preplant application or foliar applications. An additional trial conducted one year in one soil evaluated several combinations of $MnSO_4$ rates banded and a single rate of Mn-EDTA sprayed to the foliage one to four times. They reported that the combination of banded $MnSO_4$ and foliar fertilization with Mn-EDTA was the most effective treatment (maximum soybean yield was attained with lower rates than each singe application method). The authors postulated that in those Mn-deficient soils, band applications of $MnSO_4$ with the planter are effective at alleviating early Mn deficiency but additional foliar applications are needed to avert deficiencies later in the season. Studies conducted since then across the North Central region have shown mostly no soybean yield increases from Mn application, or inconsistent responses across trials and years, mainly because Mn-deficient soils are not common (Ebelhar et al., 2007; Diedrick and Mullen, 2008; Xia et al., 2009; Loecker et al., 2010; Laboski et al., 2012; Enderson et al., 2015; Mallarino, 2016; Mallarino et al., 2017; Sutradhar et al., 2017; Sharma et al., 2018).

Research in the North Central region has investigated whether soil and plant tissue testing for Mn could predict yield increase in soybean. The North-Central Extension and Research Committee for Soil Testing and Plant Analysis (NCERA-13) suggests three soil-test methods for Mn (Whitney, 2015). The test recommended by most Land Grant universities is based on extraction with 0.005 *M* DTPA (diethylenetriaminepentaacetic acid), 0.01 *M* $CaCl_2$, and 0.1 *M* triethanolamine (commonly referred to as the DTPA test). A few states recommend extraction with 0.033 *M* H_3PO_4 or 0.1 *M* HCl. The Mehlich-3 extractant is recommended and used for phosphorus (P) and potassium (K) in the region, and also is used for several micronutrients including Mn by many private laboratories although neither the NCERA-13 committee nor universities recommend it for micronutrients in region due to lack of field calibration research.

Few states in the region have soil-test interpretations for Mn and most suggested optimum levels for crops are not specific for soybean. Manganese soil-test interpretations for the tri-state region of Indiana, Ohio, and Michigan in use until earlier this year were based on the 0.1 *M* HCl test and soil pH with different interpretations for mineral or organic soils (Vitosh et al., 1995). However, a just published update of this publication does not include soil-test based interpretations for any micronutrient (including Mn) because of insufficient field calibration research to support interpretations (Culman et al., 2020). Illinois (Fernandez and Hoeft, 2009), Nebraska (Penas and Ferguson, 2000), and Missouri (Buchholz, 1983) interpretations are based on the DTPA test, and suggest that deficiencies and yield reduction are likely with less than 2 ppm in Illinois but less than 1 ppm in Nebraska and Missouri. The Wisconsin interpretations for Mn are based on the H_3PO_4 test and indicate that a value less than 10 ppm indicate deficiency and likely yield response to Mn application (Laboski and Peters, 2012). A review of published and unpublished field calibration research for Mn from 2012 until 2016 in Iowa, Kansas, and Minnesota soybean fields (Mallarino et al., 2017) showed a yield increase from Mn applied to the soil or foliage in only one of 88 trials (in a Kansas very sandy soil). Soil-test values (6-inch depth) by the DTPA test ranged from 2.3 to 59 ppm and by the Mehlich-3 test from 5 to 175 ppm. The general lack of response did not allow for determination of a critical value (the value above which no Mn fertilization would be recommended) but demonstrated a very low probability of yield response when DTPA and Mehilch-3 Mn test values are greater than 2 and 5 ppm, respectively, and a very low correlation between these two soil-test methods (r^2 0.16).

Plant-tissue testing has not been widely adopted as a basis for making micronutrient fertilizer recommendations for soybean and other commodity crops. Few Land Grant universities of the North Central region have tissue test interpretations for Mn, although most private laboratories routinely test plant tissue samples for micronutrients. As for soil tests, appropriate field calibration with yield response is needed to implement tissue testing for crop production, with special consideration of the growth stage and the plant part sampled due to well-known large variation in tissue nutrient concentrations across plant parts and time over the growing season (Vitosh et al., 1995; Fernandez and Hoeft, 2009; Enderson et al., 2015; Mallarino et al., 2017;). In the aforementioned 88 trials summarized by Mallarino et al. (2017), the Mn concentration of mature trifoliate leaves sampled at the R2 to R3 growth stage ranged from 20 to 115 ppm. The authors could not derive a Mn tissue critical value above which Mn fertilization would not be recommended due to the general lack of response, and referred to published Mn sufficiency ranges for soybean tissue of 20 to 100 ppm suggested by some states of the North Central region (Vitosh et al., 1995; Fernandez and Hoeft, 2009; Culman et al., 2020) and for no specific region of the US by Bryson et al. (2014). Application of foliar Mn at

0.2-0.5 pounds per acre in chelated form or 1-1.25 pounds per acre as a sulfate as soon as visual signs of deficiency appear, can prevent yield loss in most cases (Mallarino et al., 2017).

Glyphosate was invented in 1950 by a Swiss chemist named Dr. Henri Martin, who at the time was working for a pharmaceutical company, which saw little use for it since it had no pharmaceutical applications (Dill et at., 2010). In the process of creating water softening agents, Monsanto discovered a compound that showed herbicidal activity, which was glyphosate, and by 1974 the compound was marketed as the herbicide RoundUp® (Dill et al., 2010). The use of glyphosate was minimal until the first glyphosate tolerant crops were marketed, which started with soybean in 1996. From 1996 to 2008, glyphosate tolerant crops increased from 1.7 million to 79 million hectares and made up 70% of the world's soybean crop (Lane et al., 2012). As more glyphosate tolerant crops where developed and planted, the use of glyphosate as an herbicide increased dramatically. In 1996, the first glyphosate resistant soybean was marketed, a first-generation glyphosate resistant soybean, which used a gene from the soil bacterium *Agrobacterium spp.* strain cp4 (Zobiole., et al 2010b). Second-generation glyphosate resistant soybean varieties were first commercially marketed in 2008 (Zobiole et al., 2010b).

Field observations and research in the early 2000s began reporting reduced weed control from tank mixes of Mn fluid fertilizer and glyphosate herbicide and soybean "flash", which is a yellow-whitish coloring of the upper soybean leaves thought to be associated with use of glyphosate and reduced Mn levels with glyphosate-resistant soybean (Bailey et al., 2002; Dodd et al., 2001; Dodd et al., 2002; Huber, 2004; Bernards et al., 2005a; Benards et al., 2005b). Indiana research showed reduced Mn uptake and leaf tissue concentration in glyphosate-resistant soybean with or without applied glyphosate, and that glyphosate makes soil Mn less available or metabolically useful within the plant (Huber et al., 2004; Huber, 2007). Huber et al. (2004) further reported that glyphosate immobilized Mn when applied before, concurrent with, or within 6 to 8 days of the application of glyphosate.

Research on the use of glyphosate herbicide applied to glyphosate tolerant crops also found shifts in the soil microbial populations and that nutrients such as B, Fe, Mn, and Zn can become deficient (Thompson and Huber, 2007; Lane et al., 2012). These authors postulated that the cause of these nutrient deficiencies was alteration of the soil microbial communities by glyphosate, leading to transformation of these nutrients into forms of lower availability to plants. Glyphosate was also found to reduce the ratio of Mn reducers to Mn oxidizers in the soil by both first-generation and second-generation glyphosate resistant soybean when applied post-emergence at V2, V4, and V6 growth stages (Zobiole et al., 2010a). Recent research showed that glyphosate applied to glyphosate resistant soybean young leaves had reduced levels of Mn at twelve weeks after planting (Duke et al., 2018). Machado et al. (2019) studied effects of glyphosate on soybean foliar uptake and transport of Mn supplied as $MnSO_4$, $MnHPO_3$, Mn-EDTA, and $MnCO_3$. They reported that these Mn sources except for $MnCO_3$ increased the Mn content in leaves, the mixture with glyphosate impaired Mn transport within the plant from $MnSO_4$ and $MnHPO_3$ but not from Mn-EDTA, and saw no evidence of Mn-glyphosate complexation within the plant. They concluded that observed interferences of glyphosate with Mn uptake from foliar applications seem to be related to complexations in the tank mixture rather than by affecting the plant metabolism.

Variation in soybean genotypes seems to affect the interaction between Mn and glyphosate. Early Indiana work by Dodds et al. (2001) found that growth of a glyphosate resistant soybean variety was inhibited more severely by Mn deficiency than a conventional variety, but on a non-limiting soil there was little difference in growth between the varieties. Additional research by this group using additional varieties showed that some, but not all, glyphosate-resistant varieties were more sensitive to Mn deficiency than conventional varieties (Dodds et al. 2002). Loecker et al. (2010) evaluated soybean grain yield and the use and uptake of Mn in glyphosate resistant soybean compared to their nonresistant near-isolines at five Kansas locations. The Mn concentrations in leaf tissue were above levels considered deficient in Kansas at all sites and were not affected by the soybean genetics at any location or growth stage, but there were Mn fertilization effects on grain yield at three of the five locations. At two locations, Mn increased yield of the glyphosate-resistant varieties but not yield of the nonresistant varieties; in contrast at the other location, Mn did not increase yield of the resistant variety and did increase yield of the nonresistant variety. Therefore, the authors concluded that although soybean response to Mn is influenced by genetics, neither the Mn uptake nor the yield response to applied Mn responsiveness were conclusively affected by glyphosate-resistance. However, other research has not identified consistent differences in Mn absorption, accumulation, and availability between glyphosate-treated and non-treated glyphosate resistant soybean varieties (Bott et al. 2008; Laboski et al., 2012).

Other studies have shown no antagonism between Mn and glyphosate or inconsistent results across sites and years, although glyphosate rates higher than recommended for weed control often induced soybean "flash” symptoms and reduced yield, but the effects did not appear related to Mn levels in the soil or plant (Ebelhar et al., 2007; Gordon, 2007; Sutradhar et al., 2017). The vast majority of the 88 field trials with Mn referred to by Mallarino et al., (2017) with yield response only at one site, were done with glyphosate-resistant soybean and glyphosate herbicide but no Mn deficiency symptoms or glyphosate “flash” were observed at any site. Therefore, it is possible that overall high soil Mn levels in these states precludes not only frequent Mn deficiency, but also glyphosate-Mn interactions, and explain sometimes conflicting research results.

Today, many soybean acres are planted to glyphosate resistant soybean varieties. The use of glyphosate resistant soybean has enabled farmers to manage weeds with post emergence applications of glyphosate herbicides. Also, foliar applications of nutrients have become a large portion of fertilization for crops management in Indiana and the Corn Belt, as more products have become available and more emphasis has been placed on managing crops for higher yields. The reviewed literature provided inconclusive results regarding the potential antagonism between glyphosate and Mn fertilization, especially with foliar Mn applications. Therefore, this creative component project focused on the study of possible interactions between glyphosate and foliar fertilization with Mn impacting soybean nutrient tissue concentrations and grain yield.

MATERIALS AND METHODS

Two fields were selected for this study in 2016. One was in Adams County, Indiana, and the other was in Darke County, Ohio. The selection of the two fields was based on items such as the soil type, field variability, and field size. Equipment usage, and management practices of the current farming operation were also considered as much as possible to standardize these attributes for the test. The soil at the Ohio site (hereon referred to as OH field) consisted mainly of Blount silt loam (Aeric Epiaqualfs) with 2 to 6% slope, and the Indiana site (hereon referred to as IN field) had the same soil series but 1 to 4% slope.

Both fields were planted to corn in 2015. Both operators used a no-till system, and this management was continued for the study. Both P and K fertilizers were applied in the spring of 2016 before soybean was planted to avert any deficiency for these nutrients at 64 lb P_2O_5/acre and 132 lb K_2O/acre. A burndown herbicide application was made with a 120-ft Patriot sprayer. The burndown application consisted of 15 gal/acre of water, 2 qt of Request per 100 gal of water (a water conditioning agent), 1.125 lb/acre glyphosate (32 oz/acre Roundup Powermax), 0.0223 lb/acre of saflufenacil (1 oz/acre of Sharpen), 1.378 lb/acre of S-metolachlor and 0.328 lb/acre of metribuzin (2.1 pt/acre of Boundary 6.5EC), 0.0394 lb/acre of cloransulam-methyl (0.75 oz/acre of FirstRate), and 1 gal of MSO Premium per 100 gal of water (an adjuvant). The burndown application was performed on May 8th, 2016 for the OH field and on May 9th, 2016 for the IN field. The broadcast fertilizer application and the herbicide burndown application are both common practices for the area. Both fields were planted to Asgrow 2900RR2YD soybean. The planting depth was 1.25 inches deep in both sites. The OH field was planted on May 4th, 2016, and the IN field was planted on May 9th, 2016.

Both trials were planned for four foliar fertilization treatments arranged as a randomized complete block design (RCBD). Therefore, three blocks each with four plots were delineated at each trial. Once soybean had emerged and rows could be identified, each treatment was marked with white field sign posts. Each plot of the OH field was 45 ft wide by 367 ft long (0.38 acres), and the long side was perpendicular to the planting and herbicide spraying direction. Each plot of the IN field was 45 ft wide by 388 ft long (0.35 acres), and the long side was perpendicular to the way the plot was planted and the herbicide spraying direction.

Once plots of each trial were marked, soil samples were collected from each plot. The samples for the IN field were sampled on June 3rd, 2016 and the OH field was sampled on June 6th, 2016. Each plot was sampled in a zig-sag pattern, taking 12 cores from a depth of 6 inches (one composite soil sample per plot). Soil samples were sent to A&L Great Lakes Lab in Fort Wayne, Indiana to be tested. The tests and methods used were organic matter by loss of ignition but expressed as Walkley-Black results using a correlation by the lab, Mn by the Mehlich-3 extraction with measurement by inductively-coupled plasma (ICP), pH by the 1:1 water:soil ratio, and buffer pH by the Sikora method. Soil cation exchange capacity (CEC) was estimated as suggested by the NCERA-13 committee from extracted cations and buffer pH (Warncke and Brown, 2015). The soil test results are presented in Tables 1 and 2.

Table 1. Indiana site soil test results by plot taken prior to planting in spring 2016

Plot	Mn	pH	OM	CEC
	ppm		%	meq/100g
1	40	7.0	3.9	13.3
2	36	6.6	3.4	12.3
3	39	6.5	3.0	11.3
4	40	6.6	3.0	10.1
5	42	6.4	3.3	10.5
6	49	6.7	3.2	11.4
7	50	6.6	3.3	12.1
8	44	6.5	3.1	10.4
9	43	6.3	3.1	9.9
10	39	6.2	2.9	9.8
11	44	6.8	3.0	11.3
12	40	6.9	3.1	14.2
Average	42	6.6	3.2	11.4

Table 2. Ohio site soil test results by plot taken prior to planting in spring 2016.

Plot	Mn	pH	OM	CEC
	ppm		%	meq/100g
1	31	6.9	4.0	13.5
2	25	6.7	4.4	18.2
3	32	6.9	3.6	14.7
4	25	6.7	4.7	17.8
5	26	6.7	4.5	15.9
6	32	6.9	2.6	13.5
7	28	6.7	4.9	18.9
8	31	6.9	3.7	13.9
9	32	7.2	3.2	13.2
10	21	6.7	4.7	20.0
11	31	6.8	3.4	14.2
12	30	6.9	3.8	15.4
Average	27	6.8	4.0	15.8

A post-emergence herbicide application was made on June 19th, 2016 to both sites. The herbicide mix consisted of 15 gal/acre water, Request at 2 qt per 100 gal of water, 0.0938 lb/acre of clethodim (Tapout at 12 oz/ac), and 1 gal of oil concentrate per 100 gal of water. A John Deere utility tractor with a 3-point 15-ft sprayer was used to apply these herbicides. The herbicides were sprayed parallel to the soybean rows. Remaining weeds were physically removed on July 8th, 2016 from both sites with a garden hoe. Soybean tissue was sampled (prior to applying the treatments) at the R2 growth stage on July 11th, 2016 in both sites. Thirty of the most recently fully developed trifoliate leaves were sampled from each plot following a zigzag pattern. The leaf samples were then sent to the A&L Great Lakes Laboratory in Fort Wayne, Indiana to be tested for the total concentration of several nutrients. Tables 3 and 4 show the results of the tissue tests for both sites.

Table 3. Indiana site tissue test results by plot prior to treatment application.

Plot	N	S	P	K	Mg	Ca	B	Zn	Mn	Cu
	---------------------------------- % --------------------------------						------------------- ppm -------------------			
1	5.39	0.35	0.48	2.80	0.35	1.07	61	50	42	11
2	5.35	0.36	0.46	2.69	0.41	1.21	67	49	52	10
3	5.28	0.35	0.42	2.72	0.46	1.24	60	45	56	9
4	4.99	0.34	0.41	2.70	0.48	1.24	58	44	59	8
5	5.22	0.36	0.42	2.46	0.46	1.23	59	46	70	9
6	5.11	0.35	0.47	2.82	0.43	1.22	60	46	59	10
7	5.41	0.38	0.46	2.68	0.40	1.19	61	46	65	10
8	5.00	0.35	0.46	2.78	0.40	1.13	61	48	57	10
9	5.41	0.38	0.45	2.57	0.40	1.09	63	46	62	10
10	5.28	0.32	0.44	2.62	0.41	1.20	59	48	65	10
11	5.51	0.34	0.47	2.50	0.41	1.20	60	45	58	11
12	5.44	0.36	0.48	2.57	0.37	1.08	61	47	49	9
Average	5.28	0.35	0.45	2.66	0.42	1.18	61	47	58	10

Table 4. Ohio site tissue test results by plot prior to treatment application.

Plot	N	S	P	K	Mg	Ca	B	Zn	Mn	Cu
	---------------------------------- % --------------------------------						------------------ ppm -----------------			
1	4.61	0.31	0.38	2.76	0.36	1.06	56	36	32	9
2	4.80	0.29	0.36	2.57	0.33	1.08	57	33	34	7
3	4.61	0.30	0.41	2.96	0.31	1.10	62	36	34	9
4	4.51	0.28	0.38	2.81	0.34	1.21	56	37	33	8
5	4.72	0.33	0.42	3.05	0.34	1.14	63	39	31	9
6	4.75	0.31	0.38	2.58	0.33	1.20	58	40	44	8
7	4.97	0.32	0.39	2.48	0.37	1.09	53	35	32	8
8	4.85	0.32	0.39	2.61	0.36	1.08	58	36	31	10
9	5.09	0.32	0.39	2.51	0.39	1.07	53	36	34	10
10	4.80	0.30	0.36	2.40	0.36	1.10	51	33	30	9
11	4.99	0.34	0.44	2.59	0.53	1.06	56	38	36	9
12	4.96	0.31	0.39	2.63	0.52	1.04	53	35	30	7
Average	4.81	0.31	0.39	2.66	0.38	1.10	56	36	33	9

The foliar fertilization treatments were randomized to each replication (block) and were sprayed by trained personnel on July 16 at both sites (5 days after the plant sampling). The four treatments were water, water with the Mn treatment (Water+Mn), water with glyphosate (Water+Gly), and water with glyphosate and Mn (Water+Gly+Mn).

For the application of the foliar treatments, several different pieces of equipment were used. The sprayer was a 3-point mounted one with a 15-ft boom and TT11002 tips that were spaced 20 inches, a 75-gal tank on the back and a PTO-powered centrifugal pump. The speed of the tractor was 4 mph based on chart on the tractor. Spray pressure was 40 psi, which enable the volume applied to be 15 gal/acre. Tractor and sprayer were backed up to field edge allowing for the tractor to reach the required speed and PTO rpm before entering the treatment areas, and the sprayer boom also was engaged before leaving the field edge so that the desired volume and desired product rate were applied.

Treatments were applied to both sites by trained personnel 5 days after the plant sampling on July 16th, 2016. This date was selected because the soybean had entered the R3 stage. For the water treatment, the 75-gal tank on the sprayer boom was loaded with 50 gal of clean water by using the tank volume marks to gauge the total volume and a graduated measuring cup was used to add the 32 oz of Request, the water conditioning agent, to the tank to obtain the needed concentration. The sprayer was allowed to mix and circulate the solution for five minutes. The tractor with the 3-point sprayer boom attached to it was driven to the field and was allowed to spray for 30 s to charge booms before entering the trial to apply the Water treatment to each designated plot. After spraying all plots of this treatment, the remaining solution in the sprayer tank was measured by visually comparing marks on the 75-gallon tank to get the actual amount applied per acre.

The same general procedure was used for each of the other three treatments with their respective products, except for cleaning of the tank and sprayer system after each treatment was sprayed. The remaining solution in the sprayer tank was sprayed to a separate area of the field until empty and the sprayer was driven to the loading area where it was loaded with 20 gal of water. The tractor was then driven forward and was sped up and slowed down to rock the water back and forth in the tank before the tank was emptied. The sprayer was driven back to the loading area where it was loaded with 20 gallons of water. An amount of the next product was added to attain the desired concentration, and was agitated for 10 minutes before the tank was emptied by spraying in a field area outside the trial. This procedure was repeated one more time before loading the tank with the appropriate amounts of water and product to spray the plots of each treatment.

A second set of plant tissue samples were collected 11 days after the treatments were applied, on July 27th, 2016 at both sites using the same sampling and analyses procedures described for the pre-treatment sampling. The IN field was harvested on October 30th, 2016 with a John Deere combine with a 30-ft grain head. The combine harvested a full 30-ft swath at the center of each plot leaving 7.5 feet on each side, which acted as the buffer for the neighboring plots. After harvesting, the combine unloaded each plot into a weigh wagon and was allowed to run for 30 seconds after all grain had stopped coming out of auger. After writing down the weight of the harvested plot, two samples were taken to measure both test weight and moisture. The grain in the weigh wagon was emptied into a truck and zeroed for the next plot. The soybean grain yield was adjusted to the standard 13% moisture concentration.

Unfortunately, due to a miscommunication with the field owner, the OH field was lost because soybean was harvested across all plots before we could harvest and weigh each plot.

Therefore, for this site only the post-treatment plant tissue test results will be shown.

Analysis of variance (ANOVA) for a RCBD was performed for the post-treatment application tissue test results from both sites and grain yield data from the IN field. The ANOVA was done by the GLM procedure of the SAS statistical software (SAS Institute, 2016) for which treatments (with three degrees of freedom) and blocks (replications, with two degrees of freedom) were considered fixed effects (the residual error had 6 degrees of freedom). Least Significant Difference (LSD) was used to test differences between the four treatments only when the overall treatment main effect was statistically significant at least at $P \leq 0.10$.

RESULTS AND DISCUSSION

The soybean grain yield results for the IN field are presented in Table 5 (yield for the OH field was lost as was explained before). There were no statistically significant differences ($P \leq 0.10$) among the four treatments. No yield advantage was seen when Mn was added to either the glyphosate or the water treatment. Also, there was no change in yield from the application of glyphosate alone compared to the water application. It must be noted that the soybean yield for Plot 12, a Water treatment of 65.74 bu/acre, most likely is an outlier caused for unknown reasons because it is the highest yield for the site by 7.43 bu/acre from the second-highest yield. The Water treatment average across replications with this outlier set to missing was 53.80 bu/acre, and an ANOVA with this missed value still showed nonsignificant Mn application effects on yield (the probability was 0.40 instead of 0.16 as shown in Table 5). The lack of a glyphosate herbicide effect was expected because any weeds remaining after the preplant herbicide glyphosate application in May and the post-emergence herbicide (TapOut) application in June were removed by hand on July 8. However, a lack of effect on yield of glyphosate applied alone indicates no antagonism with soybean mineral nutrition.

Table 5. Soybean grain yield for the Indiana site.

Treatment	Block	Plot	Yield	Means	*P* ≥ F
			------- bu/acre ------		
Water	1	3	51.15	57.78	0.16
	2	7	56.45		
	3	12	65.74		
Water+Glypohsate	1	2	51.29	51.02	
	2	5	48.89		
	3	9	52.86		
Water+Mn	1	4	45.94	51.96	
	2	8	54.25		
	3	10	55.69		
Water+Glyphosate+Mn	1	1	52.61	54.52	
	2	6	52.65		
	3	11	58.31		

The lack of yield response to Mn foliar fertilization at the IN field could have been expected due to the results observed for both the soil (Table 1) and tissue tests (Table 3) from samples taken before the treatments application. The soil testing laboratory that performed the soil tests (A&L Great Lakes) used the Mehlich-3 extractant to evaluate the micronutrient levels, including Mn. The site average for Mn was 42 ppm, and results by plot showed very little variation. Neither the NCERA-13 (Whitney, 2015) nor the fertilizer guidelines from the three-state region of Indiana, Michigan, and Ohio (Culman et al., 2020) recommend the Mehlich-3 extractant for any micronutrient due to insufficient field calibration research. Enderson et al., (2015) and Mallarino et al., (2017) showed no correlation or very poor correlation between soil Mn measured with the Mehlich-3 test and the DTPA test, the latter being the one recommended for most states of the North Central region, but could not calibrate either method due to a general lack of soybean yield response. Rutgers University (New Jersey) has one of the few interpretations for soil Mn by the Mehlich-3 test in the country and nearest to the North Central region that is not specific for any crop, and test interpretation is modified according to soil pH (Heckman, 2000). For a pH range of 6.6 to 6.8 (the average pH for the IN field was 6.6, Table 1), fertilization with Mn is suggested below a critical range of 6.3 to 8.3 ppm, which is much lower than the 42-ppm level in the study. Therefore, although these critical levels may not apply to Indiana soils and the soil at the study site, the Mehlich-3 test could have assessed correctly the crop availability of Mn. In general, the soil test Mn levels from the OH field (Table 2) were lower than the IN field (Table 1) but all were much higher than Rutgers suggested critical level.

Tissue test results for the leaf samples from the IN field collected before applying the treatments (Table 3) were consistent with the observed lack of soybean yield response. No replication was expected to show a yield increase from Mn fertilization because tissue Mn averaged 58 ppm (42 to 70 ppm across plots), which is much higher than the lowest end of the Mn sufficiency range of 20 to 100 ppm referred to in the literature (Fernandez and Hoeft, 2009; Bryson et al., 2014; Mallarino et al., 2017; Culman et al., 2020). This was also the case for the OH field (Table 4).

Results by plot of leaf samples taken at the R3 growth stage, 11 days after application of treatments, at both locations are shown in Tables 6 and 7. Visual observations of the values for the different nutrients show approximately similar magnitudes of variability within or across blocks to that observed for the sampling before the treatments application.

Table 6. Indiana site tissue test results one week after treatments application.

Treatment†	Block	Plot	N	S	P	K	Mg	Ca	B	Zn	Mn	Cu
			%						ppm			
Water	1	3	4.44	0.32	0.36	2.28	0.43	1.66	67	50	75	9
Water	2	7	4.97	0.34	0.41	2.54	0.39	1.51	71	49	80	10
Water	3	12	5.11	0.36	0.41	2.22	0.34	1.45	71	50	59	8
W+G	1	2	4.92	0.37	0.44	2.39	0.34	1.41	69	55	57	10
W+G	2	5	5.12	0.35	0.38	2.04	0.43	1.53	70	52	77	10
W+G	3	9	5.14	0.37	0.4	2.26	0.37	1.42	72	54	86	9
W+Mn	1	4	4.86	0.33	0.38	2.45	0.43	1.57	68	55	82	8
W+Mn	2	8	4.57	0.35	0.37	2.43	0.39	1.58	76	58	90	9
W+Mn	3	10	5.07	0.36	0.37	1.98	0.39	1.41	68	50	76	7
W+G+Mn	1	1	5.13	0.4	0.44	2.51	0.34	1.31	69	54	51	10
W+G+Mn	2	6	5.11	0.31	0.37	2.19	0.36	1.31	66	50	90	8
W+G+Mn	3	11	5.13	0.35	0.42	2.33	0.44	1.55	74	55	88	8

† W+G, water with glyphosate; W+Mn, water with Mn; W+G+Mn, water with glyphosate and Mn.

Table 7. Ohio site tissue test results one week after treatments application.

Treatment†	Block	Plot	N	S	P	K	Mg	Ca	B	Zn	Mn	Cu
			%						ppm			
Water	1	3	5.19	0.33	0.41	2.35	0.36	1.33	67	46	34	11
Water	2	7	5.31	0.34	0.38	2.38	0.39	1.42	61	44	42	11
Water	3	12	5.31	0.35	0.38	2.31	0.4	1.41	63	50	33	15
W+G	1	2	5.13	0.34	0.37	2.32	0.37	1.5	66	43	46	12
W+G	2	5	5.38	0.34	0.38	2.39	0.36	1.35	65	46	33	11
W+G	3	9	4.96	0.3	0.39	2.26	0.44	1.49	63	44	38	10
W+Mn	1	4	5.19	0.33	0.38	2.31	0.36	1.44	66	49	37	12
W+Mn	2	8	5.19	0.32	0.36	2.18	0.37	1.39	63	44	43	10
W+Mn	3	10	5.13	0.34	0.38	2.06	0.48	1.48	61	43	43	11
W+G+Mn	1	1	5.17	0.36	0.37	2.29	0.39	1.32	61	44	41	11
W+G+Mn	2	6	5.21	0.35	0.39	2.32	0.32	1.33	70	47	34	11
W+G+Mn	3	11	5.15	0.33	0.38	2.16	0.48	1.51	55	42	42	11

† W+G, water with glyphosate; W+Mn, water with Mn; W+G+Mn, water with glyphosate and Mn.

Table 8 shows the tissue test means across replications for both sites. There were no statistically significant effects ($P \leq 0.10$) of Mn fertilization or glyphosate application on tissue test concentrations for any nutrient, except for difficult to explain differences for P at the OH field, which could have resulted from random variability. A lack of Mn tissue concentration response is not surprising because foliar fertilization research often has shown infrequent or inconsistent increases of nutrient concentrations in the tissue, mainly when there is no yield response (Enderson et al., 2015). A foliar applied nutrient could increase the leaf concentration because the nutrient still has not been translocated or, conversely, may not affect or could decrease it because of dilution with new growth.

Table 8. Treatment means for soybean tissue test results one week after spraying the treatments application for the Indiana and Ohio sites.

State	Treatment†	N	S	P	K	Mg	Ca	B	Zn	Mn	Cu
		------------------------------ % ---------------------------						--------------- ppm --------------			
IN	Water	4.84	0.34	0.39	2.35	0.39	1.54	70	50	71	9
	W+G	5.06	0.36	0.41	2.23	0.38	1.45	70	54	73	10
	W+Mn	4.83	0.35	0.37	2.29	0.40	1.52	71	54	83	8
	W+G+Mn	5.12	0.35	0.41	2.34	0.38	1.39	70	53	76	9
	Statistics‡	0.29	0.76	0.46	0.86	0.93	0.47	0.98	0.28	0.75	0.14
OH	Water	5.20	0.35	0.38ab	2.24	0.42	1.41	61	44	42	11
	W+G	5.12	0.33	0.40a	2.31	0.37	1.38	67	46	35	11
	W+Mn	5.21	0.34	0.37b	2.27	0.38	1.43	64	46	41	12
	W+G+Mn	5.24	0.33	0.38ab	2.29	0.40	1.43	62	46	37	11
	Statistics	0.56	0.60	0.05	0.87	0.19	0.78	0.20	0.82	0.39	0.54

† W+G, water with glyphosate; W+Mn, water with Mn; W+G+Mn, water with glyphosate and Mn.
‡ Statistics, probability of the treatments main effect ($P \geq F$). Numbers in a column with similar letters do not differ at $P \leq 0.10$.

CONCLUSIONS

Neither glyphosate, Mn, or the combination of both affected soybean yield when applied during the late R2 growth stage at the Indiana trial. Unfortunately, yield could not be measured at the Ohio trial. The finding for the Indiana trial supports results of most previous studies across the North Central region that found no soybean response to Mn fertilization, although some of the responsive instances were in Indiana. No visual symptoms of glyphosate phytotoxicity or Mn deficiency were observed in soybean at either location.

Tissue test results of soybean trifoliate leaves sampled at the R2 growth stage from both sites were aligned with the yield results from the Indiana location. These tissue results indicated that the probability of a response from Mn at both sites was very low because were within the sufficiency level range suggested in the literature. Tissue tests of soybean trifoliate leaves sampled at the R3 growth stage (11 days after the treatments application) from both sites show no effect of the Mn application on the concentration of Mn or any other micronutrient. This result agrees with previous research indicating that foliar fertilization of soybean with Mn seldom increases the tissue concentration the Mn and other micronutrients when there is no yield response.

Overall, the study showed no antagonism or interaction between glyphosate and soybean Mn nutrition, which agrees with results of many trials conducted across the North Central region when the crop availability of soil Mn is optimum or higher for soybean and there is no yield response to applied Mn.

REFERENCES

Alejandro, S., S. Höller, B. Meier, and E. Peiter. 2020. Manganese in Plants: From Acquisition to Subcellular Allocation. Frontiers in Plant Science 11:300. doi: 10.3389/fpls.2020.00300.

Alt, D., S.J. Ng, J. Grusenmeyer, and L.E. Lindsey. 2018. Seed yield and quality of transgenic high-oleic and conventional soybean as influenced by foliar manganese Application. Crop Science, 58:874-879.

Bailey, W.A., D.H. Poston, H.P. Wilson, and T.E. Hines. 2002. Glyphosate interactions with manganese. Weed Technology, 16:792-799.

Benards, M.L., K.D. Thelen, and D. Penner. 2005a. Glyphosate efficacy is antagonized by manganese. Weed Technology, 19:27-34.

Benards, M.L., K.D. Thelen, D. Penner, R.B. Muthukumaran, and J.L. McCraken. 2005b. Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation. Weed Science, 53:787-794.

Bott, S., T. Tesfamariam, H. Candan, I. Cakmak, V. Römheld, and G. Neumann. 2008. Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (*Glycine max* L.). Plant and Soil, 312:185-194.

Buchholz, D.D. 1983. Soil test interpretations and recommendations handbook. Department of Agronomy. Univ. of Missouri, Columbia.

Enderson, J.T., A.P. Mallarino, and M.U. Haq. 2015. Soybean yield response to foliar-applied micronutrients and relationships among soil and tissue tests. Agronomy Journal, 107:2143-2161.

Culman, S., A. Fulford, J. Camberato, and K. Steinke. 2020. Tri-state fertilizer recommendations for corn, soybeans, wheat, and alfalfa. Bulletin 974. College of Food, Agricultural, and Environmental Sciences. The Ohio State University. Columbus, OH.

Diedrick, K.A., and R.W. Mullen. 2008. Foliar manganese and glyphosate formulation yield effects on glyphosate resistant soybeans in Ohio. Proc. North Central Extension-Industry Soil Fertility Conf. 24:66-69.

Dill, G.M., R. D. Sammons, P.C. Feng, F. Kohn, K. Kretzmer, A. Mehrsheikh, M. Bleeke, J. L.

Honegger, D. Farmer, D. Wright, and E. A. Haupfear. 2010. Discovery, Development, Applications, and Properties. p. 1-33. In V.K. Nandula (Ed.). Glyphosate resistance in crops and weeds: History, Development, and Management. Hoboken, NJ. John Wiley & Sons, Inc. https://onlinelibrary.wiley.com/doi/book/10.1002/9780470634394.

Dodds, D.M., M.V. Hickman, and D.M. Huber. 2001. Comparison of micronutrient uptake by glyphosate resistant and non-resistant soybeans. p. 96. In Proceedings, Vol. 56, North Central Weed Science Society.

Dodds, D.M., Huber, D.M. and M.V. Hickman. 2002. Micronutrient levels in normal and glyphosate-resistant soybean varieties. p. 107. In Proceedings, Vol. 57. North Central Weed Science Society.

Duke, S.O., A.M. Rimando, K.N. Reddy, J.V. Cizdziel, N. Bellaloui, D.R. Shaw, M.M. Williams II, and J.E. Maul. 2018. Lack of transgene and glyphosate effects on yield, and mineral and amino acid content of glyphosate-resistant soybean. Pest Management Science, 74:1166-1173.

Ebelhar, S.A., E.A. Adee, and C.D. Hart. 2007. Soil pH and manganese effects on roundup ready® soybeans. p. 88-101. In Proc. North Central Extension-Industry Soil Fertility Conference, Des Moines, IA. Vol. 23. 14–15 Nov. 2007. Int. Plant Nutrition Inst., Brookings, SD.

Fernandez, F.G., and R.G. Hoeft. 2009. Managing soil pH and crop nutrients. In Illinois agronomy handbook. Univ. of Illinois. http://extension.cropsciences.illinois.edu/handbook.

Gaspar, A.P., C.A.M. Laboski, S.L. Naeve, and S.P. Conley. 2018. Secondary and micronutrient uptake, partitioning, and removal across a wide range of soybean seed yield levels. Agronomy Journal, 110:1328-1338.

Gordon, W.B. 2007. Does glyphosate gene affect manganese uptake in soybeans? Fluid Journal, 15(2):12-13.

Heckman, J.R.2000. Manganese need of soil and crops in New Jersey. Rutgers Cooperative Research & Extension Fact Sheet. FS973.

Huber, D. M., J. D. Leuck, W. C. Smith, and E. P. Christmas. 2004. Induced manganese deficiency in GM soybeans. p. 80-83. In Proc. North Central Ext.-Ind. Soil Fert. Conf. Vol. 20:80-83. Int. Plant Nutrition Inst., Brookings, SD.

Huber, D.M. 2007. What about glyphosate-induced manganese deficiency? Fluid Journal, 15(4):20-22.

Laboski, C.A.M., J.B. Peters. 2012. Nutrient Application Guidelines for Field, Vegetable, and Fruit Crops in Wisconsin. University of Wisconsin Extension Cooperative Extension.

Laboski, C.A.M., T. Andraski, S. Conley, and J. Gaska. 2012. Effect of soybean variety, glyphosate use, and manganese application on soybean yield. Proc. Wisconsin Crop Management Conf. 51:49-64.

Lane, M., N. Lorenz, J. Saxena, C. Ramsier, and R.P. Dick. 2012. Microbial activity, community structure and potassium dynamic in rhizosphere soil of soybean plants treated with glyphosate. Pedobiologia - International Journal of Soil Biology, 55:153-159.

Loecker, J.L., N.O. Nelson, W.B. Gordon, L.D. Maddux, K.A. Janssen, and W.T. Schapaugh. 2010. Manganese response in conventional and glyphosate resistant soybean. Agronomy Journal, 102:606-611.

Machado, B.A., M.H. Gomes, J.P. Marques, R. Otto, and H.W. Carvalho. 2019. X-ray spectroscopy fostering the understanding of foliar uptake and transport of Mn by soybean (*Glycine max* L. Merril): Kinetics, chemical speciation and effects of glyphosate. Journal of Agricultural and Food Chemistry, 1-36.

Mallarino, A.P. 2016. Value of tissue testing to improve phosphorus, potassium, and micronutrients management for corn and soybean. p. 139-146. In The Integrated Crop Management Conf. Proceedings. Nov. 30-Dec. 1, 2016. Iowa State Univ. Ext.

Mallarino, A.P., D.E. Kaiser, D.A. Ruiz-Diaz, C.A. Laboski, J.J. Camberato, and T.J. Vyn. 2017. Micronutrients for soybean production in the North Central region. Iowa State Univ. Ext. CROP 3135 (rev.). https://store.extension.iastate.edu/Product/15259.

Mengel, K., H. Kosegarten, E.A. Kirkby, and T. Appel. 2001. Principles of plant nutrition. Kluwer Academic Publ., Dordrecht, the Netherlands.

Mortvedt, J.J., F.R. Cox, L.M. Shuman, and R.M. Welch, editors. 1991. Micronutrients in agriculture. 2nd ed. SSSA, Madison, WI.

Penas, E.J., and R.B. Ferguson. 2000. Micronutrients. In: R.B. Ferguson, and K.M. DeGroot, editors, Nutrient management for agronomic crops in Nebraska. EC155. University of Nebraska-Lincoln Extension.

SAS Institute. 2011. The SAS system for windows. Version 9.3. SAS Inst., Cary, NC.

Sharma, S., S. Culman, A. Fulford, L. Lindsey, D. Alt, and G. Looker. 2018. Corn, soybean, and alfalfa yield responses to micronutrient fertilization in Ohio. AGF-159. College of Food, Agricultural, and Environmental Sciences. The Ohio State University. Columbus, OH. https://ohioline.osu.edu/factsheet/agf-519.

Shacklette, H.T., and J.C. Boerngen. 1984. Element concentrations in soils and other surficial materials of the conterminous United States. U.S. Geological Survey. Professional Paper

1270. U.S. Gov. Print. Office, Washington, D.C.

Scott, W.O., and S.R. Aldrich. 1970. Modern soybean production. p. 84-85 In The Farm Quarterly. Cincinnati, OH.

Sutradhar, A.K., D.E. Kaiser, and L.M. Behnken. 2017. Soybean response to broadcast application of boron, chlorine, manganese, and zinc. Agronomy Journal, 109:1048-1059.

Thompson, I.A. and D.M. Huber. 2007. Manganese and plant disease. p. 139-153. In Datnoff, L.E., Elmer, W.E. and D.M. Huber. (Eds.) Mineral nutrition and plant disease. Amer. Phytopathology Society, St. Paul, MN.

Vitosh, M.L., J.W. Johnson, and D.B. Mengel. 1995. Tri-state fertilizer recommendations for corn, soybeans, wheat, and alfalfa. Ext. Bull. E-2567. Michigan State University Extension, East Lansing, MI.

Warncke, D., and J.R. Brown. 2015. Potassium and other basic cations. In Recommended chemical soil test procedures for the North Central Region. p. 7.1-7.3 In North Central Regional Publ. No. 221 (Rev 2015). Missouri Agricultural Experiment Station SB 1001. https://extensiondata.missouri.edu/pub/pdf/specialb/sb1001.pdf.

Whitney, D.A. 2015. Micronutrients: Zinc, iron, manganese and copper. In Recommended chemical soil test procedures for the North Central Region. p. 9.1-9.4 In North Central Regional Publ. No. 221 (Rev 2015). Missouri Agricultural Experiment Station SB 1001.

Xia, Y., J.J. Camberato, and T.J. Vyn. 2009. Effects of glyphosate application and manganese fertilization on leaf manganese concentration and yield of glyphosate resistant soybean. Proc. North Central Extension-Industry Soil Fertility Conf. 25:147-154.

Zobiole, L.H., R.J. Kremer, R.S. Oliveira Jr., and J. Constantin. 2010a. Glyphosate affects microorganisms in rhizosphere of glyphosate-resistant soybeans. Journal of Applied Microbiology, 110:118-127.

Zobiole, L.H., R.J. Kremer, R.S. Olveira Jr, and J. Constantin. 2010b. Glyphosate affects photosynthesis in first and second generation of glyphosate-resistant soybeans. Plant and Soil, 336:251-256.

Zobiole, L.H., R.S. Oliveira Jr., D.M. Huber, J. Constantin, C. Castro, F.A. Oliveira, and A. Oliveira Jr. 2010c. Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. Plant and Soil, 328:57-69.